SUPPLEMENTARY TABLES S1, S2 AND S3

Supplementary Table S1

Plasmids constructed for this work.

Plasmid	Description	Cloning strategy
pHD 1147	*CAT-PGKB* reporter transcribed by RNA Pol I	Quijada, L., et al. (2002) *Nucleic Acids Res.*, **30**, 1-11.
pHD 1343	*CAT-GC-EP1* reporter transcribed by T7 RNA polymerase	Irmer, H. and Clayton, C.E. (2001) *Nucleic Acids Res.*, **29**, 4707-4715.
pHD 1839	*PAN2/V5 in situ* tag	The UTR part was amplified with the forward primer 5'-aattccgcggttcttcattttgatcttgtatcttc-3' and with the reverse primer 5'-atattctagattatacgctgccaatacccagtgcag-3', inserted with SacII and XbaI into the Bla/V5 vector The coding region part (accession number Tb06.28P18.500) was amplified with the forward primer 5'-aattctcgagagcgttccgtggaaggttcgaggta-3' and the reverse primer 5'-atatgggcccaatacatgcactgcgcaccgcc-3', inserted with ApaI and XhoI into the Bla/V5 vector
pHD 1843	CAF1 RNAi	Part of the *CAF1* ORF (accession number Tb06.3D8.300) was amplified using forward primer 5'-gaggatcctctgcctcacagaggatgtg-3' and reverse primer 5'-cgggatcccgaggaatcttcacagagcc-3', PCR product was cloned into p2T7$^{TA\ blue}$
pHD 1845	PAN3 RNAi	Part of the *PAN3* ORF (accession number Tb11.01.5540) was amplified using forward primer 5'-gaggatccttgtttcggcaatatcgtca-3' and reverse primer 5'-cgggatccactgcgaaatcaagcgatct-3', PCR product was cloned into p2T7$^{TA\ blue}$
pHD 1851	*PAN2* stuffer RNAi	Part of the *PAN2* ORF was amplified using forward primer 5'-gagaagatctgcatgccgtcgttatcgtgacccttt-3' and reverse primer 5'-cggaattcgtcgactggtgcgagtttgtctcttg-3'. The first insert was cloned with SalI and SpeI into pHD1144. After that the second insert was cloned with EcoRI and BglII into *PAN2* insert1/pHD 1144. The *PAN2* stuffer was cut out of *PAN2* insert1/ *PAN2* insert2/1144 with HindIII and BglII. pHD 1145 was linearised with HindIII and BamHI and the *PAN2* stuffer was inserted.
pHD1905	Tb927.5.1270 RNAi	A fragment of the Tb927.5.1270 gene was amplified using **CGAAGATCTGCATGCCCACAGGAGGAGGTAACCAA and CGGAATTCGTCGACcatcatcatcatcatc then cloned to give a stem-loop into** pHD1145 (1907 is in pHD1146)
pHD1906	Tb11.22.0004 (MEX67) RNAi	A fragment of the Tb11.22.0004 gene was amplified using **GGAAGATCTGCATGCTGTTAAACCCACTGGAAGGC and CGGAATTCCTGCAGAACACACGAGTGAAGTTGCG then cloned to give a stem-loop into** pHD1145 (1908 is in pHD1146)

Supplementary Table S2

Cloned 5’ and 3’ ends from circularised *RPL37A* mRNA (encoded by locus Tb10.100.0110). “Position on SL” denotes the furthest upstream *SL* nucleotide: A full-length capped mRNA stops at nt 0 and one with only one nt of the *SL* would stop at nt 39. “Nt before ATG” is the length of the 5’ UTR plus *SL*. The full-length clones were obtained from the 300nt band. For sequences see Supplementary Figure S4.
“TAP decap” indicates removal of the cap with tobacco acid pyrophosphatase. This digestion neither reduced the number of PCR cycles required to obtain detectable products, nor influenced the structures of the products obtained. We suspect that this is because the four methylated nucleotides proximal to the cap at the 5'-end of the trypanosome spliced leader inhibit reverse transcriptase {Freistadt, 1988 #1748;Freistadt, 1987 #1749;Perry, 1987 #1750;McNally, 1992 #691;Ullu, 1991 #907}. To solve this problem, we removed the 5’-end of the spliced leader using a specific oligonucleotide and RNase H.
Lanes denoted “N” were made using a nested PCR, the others with a single primer pair. If two lanes have the same clone number (a and b) then the clone included an open reading frame, with different amplified ends at the extremities (3'-UTR - 5'-UTR - ORF - 3'-UTR - 5'-UTR). This could happen if reverse transcriptase had read right around the circle, then incomplete PCR products originating from different mRNAs hybridised within the ORF during the PCR reaction.
#In these case sequencing was only possible from one end, and terminated in the poly(A), so the number of A's present may be greater than indicated. It is also possible that the oligos were deleted during plasmid replication.
Although a few non-A residues were found in the poly(A) tails, these were no more frequent than nucleotide changes within the rest of the sequences so were probably PCR artefacts (Supplementary Figure S4).

Clone*	TAP decap	nt of SL	Nt before ATG	Nt after stop codon (no Poly A)	Poly A Tail length
Full length		39	55	108-159	
XRNA+, RNase H					
R-3-1	-	24	41	127	31
R-3-2	-	21	38	136	35
R-3-3	-	26	43	108	36
R-3-4	-	18	35	136	37
R-3-5	-	18	35	108	42
XRNA+					
4-4Na	+	7	24	108	32
4-6	+	7	24	108	37
3-1a	-	24	41	108	31
3-1N	-	8	25	108	5
3-2	-	8	25	108	48
3-2N	-	8	25	108	41
3-3a	-	-	13	108	41
3-3N	-	8	25	136	62
3-4a	-	7	24	108	50
3-5	-	7	24	108	93
4-1Na	+	8	25	47	-
4-1Nb	+	8	25	24	-
4-2Na	+	11	28	83	-
4-2Nb	+	34	51	65	-
4-3	+	8	25	91	-
4-3N	+	29	46	10	-
4-4Nb	+	8	25	83	-
4-5Na	+	11	28	83	-
4-5Nb	+	7	24	11	-
3-4Na	-	8	25	30	-
3-4Nb	-	10	27	6	-
3-5Na	-	8	25	140	-
3-5Nb	-	8	25	23	-
XRNA RNAi, RNase H					
R-1-1	-	26	43	108	33
R-1-2	-	24	41	136	33
R-1-3	-	26	43	136	32
R-1-4	-	18	35	136	43
R-1-5	-	18	35	108	39
XRNA RNAi					
1-2N	-	-	4	136	26
1-3	-	7	24	108	32
1-3Na	-	-	6	108	5
1-3Nb	-	-	-7	108	39
1-5N	-	?		131	35#
1-7	-	-	1	109	10
2-1Na	+	-	10	136	22
2-2a	+	7	24	108	34
2-3a	+	7	24	159	112
2-5Nb	+	-	6	108	29
1-1N	-	9	26	-	-
1-4Na	-	29	46	85	-
1-4Nb	-	11	28	144	-
2-1Nb	+	20	37	70	-
2-2Na	+	9	26	42	-
2-2Nb	+	-	0	29	-
2-3Na	+	20	37	35	-
2-3Nb	+	11	28	97	-
2-4a	+	24	41	63	-
2-5Na	+	8	25	71	-

Supplementary Table S3

Cloned 5' and 3' ends from circularised *EP* mRNA. Clones from all different *EP* loci are included. Clones designated A and B are from two independent experiments. For experiment B, "a" denotes clones from nested PCR "a" (Figure 7C) and "b" from PCR "b". "Sequence no" refers to the number of the sequence in Figure S6.

For A and B, the first PCR was done with primers RT and 31 and the second with primers 52 and 32 " (Figure 7C). The destabilising 26mer is at nt 134-159 of the 3'-UTR, so is present in 3' fragments of 159 nt and above. *** primary transcript, retains the transcription start site.

Clone	Sequence no	TAP decap	nt of SL	nt 5' to ATG	nt 3' to stop	p(A)
Full-length			39	70	297	
XRNA+, RNase H						
Bb-3-2	EP-3-2	-	26	58	17	10
Ba-3-1	pEP-3-1	-	25	57	13	3
Ba-3-2	pEP-3-2	-	25	57	57	11
Ba-3-3	pEP-3-3	-	-	-13	22	4
Ba-3-4	pEP-3-4	-	29	61	-4	6
Bb-3-4	EP-3-4	-	24	56	-5	-
Bb-3-5	EP-3-5	-	24	56	38	-
Ba-3-5	pEP-3-5	-	26	58	39	-
XRNA+, no RNase H						
Ba-4-1	pEP-4-1	-	***	115	40	4
Ba-4-2	pEP-4-2	-	***	115	63	3
A-3-2	EP1-3-2	-	26	58	35	11
A-3-4	EP1-3-4	-	25	57	13	12
A-3-6	EP1-3-6	-	34	66	3	17
A-4-2	EP1-4-2	+	26	58	105	17
A-4-3	EP1-4-3	+	26	58	-14	5
A-4-6	EP1-4-6	+	-	-13	53	4
Bb-4-1	EP-4-1	-	26	58	4	3
Bb-4-2	EP-4-2	-	9	41	43	7
Bb-4-3	EP-4-3	-	25	57	36	12
Bb-4-5	EP-4-5	-	-	20	17	28
Ba-4-5	pEP-4-5	-	29	61	36	12
A-3-1	EP1-3-1	-	-	-46	55	-
A-3-3	EP1-3-3	-	-	0	22	-
A-3-5	EP1-3-5	-	27	59	-10	-
A-4-1	EP1-4-1	+	-	17	16	-
A-4-4	EP1-4-4	+	28	60	1	-
A-4-5	EP1-4-5	+	-	-16	-14	-
Bb-4-4	EP-4-4	-	8	40	122	-
Ba-4-3	pEP-4-3	-	27	59	-1	-
Ba-4-4	pEP-4-4	-	29	62	4	-
XRNA RNAi, RNase H						
Bb-1-4	EP-1-4	-	26	58	16	10
Bb-1-2	EP-1-2	-	23	55	19	-
Bb-1-3	EP-1-3	-	26	58	-12	-
Ba-1-1	pEP-1-1	-	26	58	-4	-
Ba-1-2	pEP-1-2	-	26	58	31	-
Ba-1-3	pEP-1-3	-	27	59	4	-
Ba-1-4	pEP-1-4	-	26	58	-4	-
Ba-1-5	pEP-1-5	-	24	56	55	-
XRNA RNAi, no RNase H						
A-1-3	EP1-1-3	-	-	-48	40	5
A-1-5	EP1-1-5	-	-	0	-8	5
A-2-5	EP1-2-5	+	-	-24	0	4
A-1-2	EP1-1-2	-	-	0	28	-
A-1-4	EP1-1-4	-	-	-4	53	-
A-1-6	EP1-1-6	-	-	13	14	-
A-2-1	EP1-2-1	+	-	-31	65	-
A-2-2	EP1-2-2	+	-	21	135	-
A-2-3	EP1-2-3	+	-	0	24	-
A-2-4	EP1-2-4	+	-	-50	121	-
Bb-2-3	EP-2-3	-	-	-37	31	-
Ba-2-1	pEP-2-1	-	-	0	27	-
Ba-2-2	pEP-2-2	-	-	-29	299	-
Ba-2-3	pEP-2-3	-	-	-20	301	-
Ba-2-4	pEP-2-4	-	.	-23	102	-
Ba-2-5	pEP-2-5	-	26	58	-14	-

Supplementary Table S4

Cloned 5' and 3' ends from circularised *EP* mRNA. Clones designated Y and Z are from independent experiments. The first PCR was done with primers RT and 32. For experiment Y, the second PCR was with primers 51 and 33, and for Z with primers 51 and 34. Note that these results are not representative because we deliberately selected clones with longer inserts for sequencing. Statistical analysis would therefore not be appropriate.

*wild-type polyadenylation site; #ends with aat or aaat instead of aaaat. $ends with poly(T)

Clone	3' primer	SL	nt 5' to ATG	nt 3' to TAA	p(A)
Full-length		39	70	297	
XRNA+, RNase H					
Y-3-1	33	27	60	180	-
Y-3-2	33	27	60	130	-
Y-3-3	33	30	63	178	-
Z-3-1	34	24	57	280	-
Z-3-4	34	24	57	275	-
Z-3-5	34	26	59	239	6
XRNA+, no RNase H					
Y-4-1	33	-	-49	144	-
Y-4-2	33	18	42	105	-
Y-4-3	33	27	60	99	-
Y-4-4	33	-	-23	145	-
Y-4-5	34	-	-19	115	-
Z-4-1	34	-	32	-	-
Z-4-2	34	-	-24	295	-
Z-4-3	34	-	14	273	-
Z-4-4	34	-	-7	306	6
Z-4-5	34	-	-23	294	-
Z-4-6	34	-	-3	284	-
XRNA RNAi, RNase H					
Y-1-1	33	-	1	291	-
Y-1-2	33	27	60	136	-
Y-1-3	33	26	59	92	5
Y-1-4	33	26	59	137	-
Y-1-5	33	26	59	-	-
Z-1-1	34	-	32	-	-
Z-1-2	34	-	-	297	#
Z-1-3	34	-	-10	293	-
Z-1-4	34	-	1	297	#
Z-1-5	34	-	-22	286	-
Z-1-7	34	-	0	297	#
XRNA RNAi, no RNase H					
Y-2-1	33	-	-28	119	-
Y-2-2	33	-	-25	113	-
Y-2-3	33	-	-26	96	-
Y-2-4	33	-	-26	96	-
Y-2-5	33	-	-26	96	-
Z-2-1	34	-	-27	*297	$
Z-2-2	34	-	-27	273	-
Z-2-3	34	-	-26	-	-
Z-2-4	34	-	-18	291	-
Z-2-5	34	-	1	254	-